Cytotoxic T-Lymphocyte Antigen-4 Single Nucleotide Polymorphisms Are Not Associated with Outcomes after Unrelated Donor Transplantation: A Center for International Blood and Marrow Transplant Research Analysis

Salyka Sengsayadeth [1,*], Tao Wang [2], Stephanie J. Lee [3,4], Michael D. Haagenson [4], Stephen Spellman [4], Marcelo A. Fernandez Viña [5], Carlheinz R. Muller [6], Michael R. Verneris [7], Bipin N. Savani [1], Madan Jagasia [1]

[1] *Section of Hematology and Stem Cell Transplantation, Vanderbilt-Ingram Cancer Center, Nashville, Tennessee*
[2] *Division of Biostatistics, Medical College of Wisconsin, Milwaukee, Wisconsin*
[3] *Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington*
[4] *Center for International Blood & Marrow Transplant Research, National Marrow Donor Program, Minneapolis, Minnesota*
[5] *Department of Pathology, Stanford Medical School, Stanford University, Stanford, California*
[6] *Germany National Bone Marrow Registry, Ulm, Germany*
[7] *Blood and Marrow Transplant Program, Department of Medicine, University of Minnesota, Minneapolis, Minnesota*

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A B S T R A C T

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) plays an essential role in T cell homeostasis by restraining immune responses. AG and GG genotypes of donor *CTLA-4* SNP rs4553808 in patients after unrelated donor hematopoietic stem cell transplantations (HSCT) have been shown to be an independent predictor of inferior relapse-free survival (RFS) and overall survival (OS) compared with those with the AA genotype, in single-center studies. We tested the hypothesis that SNP rs4553808 is associated with RFS, OS, nonrelapse mortality (NRM) and the cumulative incidence of acute graft-versus-host disease (GVHD) and chronic GVHD in adults with acute myeloid leukemia and advanced myelodysplastic syndrome undergoing a first 8/8 or 7/8 HLA-matched unrelated donor HSCT. Multivariable analysis adjusting for relevant donor and recipient characteristics showed no significant association between SNP rs4553808 and OS, RFS, NRM, and incidence of acute and chronic GVHD. An exploratory analysis of other *CTLA-4* SNPs, as well as studying the interaction with antithymocyte globulin, also demonstrated no significant associations. Our results indicate that *CTLA-4* SNPs are not associated with HSCT outcomes.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is curative therapy for many malignant hematologic diseases. However, the success of HSCT is often limited by disease relapse and graft-versus-host disease (GVHD). There are currently no established biologic predictors of transplantation outcomes. Understanding how to achieve the optimal balance between a potent graft-versus-tumor (GVT) effect and minimizing the risk of GVHD remains elusive. Both GVT and GVHD are dependent on maintaining T cell homeostasis, which requires regulation of an intricate system of immunologic checks and balances. One of the regulatory molecules that serves as an important immunologic checkpoint is cytotoxic T-lymphocyte antigen−4 (CTLA-4), which primarily functions in inhibition of T cell activation. CTLA-4 is a member of the immunoglobulin superfamily, and it, along with other regulatory molecules, such as programmed death-1 (PD-1), plays an important role in regulation of peripheral tolerance [1]. Upon recognition of an antigen by the T cell receptor, CTLA-4 competes with CD28 to bind with CD80/86. This signal, in turn, leads to inhibition of downstream T cell activation and subsequently down-regulates the immune response [2]. In clinical practice, this mechanism of action has been harnessed as immunotherapy, exemplified by the drug ipilimumab, a CTLA-4−blocking monoclonal antibody that augments the immune response and improves overall survival (OS) in patients with metastatic melanoma [3].

The impact of SNPs of *CTLA-4* has previously been demonstrated in solid tumors. Specifically, the G allele of rs4553808 has been associated with a positive response to ipilimumab in patients with metastatic melanoma [4]. In HSCT studies, *CTLA-4* SNPs have been associated with differences in relapse-free survival (RFS), OS, and GVHD, but with discordant results from various investigators [5-9]. Previously, we have shown that donor SNP rs4553808 is an

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* Correspondence and reprint requests: Salyka Sengsayadeth, MD, Vanderbilt-Ingram Cancer Center, 1301 Medical Center Drive, Nashville, TN 37232.
E-mail address: salyka.sengsayadeth@vanderbilt.edu (S. Sengsayadeth).

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independent pretransplantation predictor of outcomes in unrelated donor HSCT, with patients who receive transplants from donors with AG or GG genotypes having inferior RFS and OS compared with those receiving transplants from donors with the AA genotype [10]. We hypothesized that these results could be validated in a larger, more homogenous cohort of patients and, if validated, could be basis for pretransplantation donor *CTLA-4* genotyping as a risk-stratification tool that would aid in prediction of relapse risk. Patients at high risk of relapse could be specifically targeted for peri-transplantation immunomodulation or preemptive therapies to reduce the risk of relapse.

Additionally, the interaction of *CTLA-4* SNPs with use of antithymocyte globulin (ATG) was also studied. In patients undergoing a matched unrelated donor HSCT, ATG is often used as part of GVHD prophylaxis. The efficacy of ATG in prophylaxis of GVHD is dependent on its ability to deplete T-lymphocytes of the graft [11]. We hypothesized that genetic variation in *CTLA-4* may interact with the utilization of the immunomodulatory drug ATG as part of the conditioning regimen in unrelated donor transplantations. If an interaction was observed, then that this could additionally facilitate personalization of GVHD prophylaxis.

STUDY DESIGN

This study was completed in collaboration with the Center for International Blood and Marrow Transplant Research (CIBMTR) and the National Marrow Donor Program (NMDP). The CIBMTR is an association of more than 500 transplantation centers worldwide that collects statistical data and maintains longitudinal follow-up on consecutive allogeneic HSCTs. Data are submitted to the Statistical Center at the Medical College of Wisconsin, and quality is ensured with computerized checks, physician review of data, and onsite audits of centers. Observational data collection by CIBMTR was approved by the Institutional Review Board of the NMDP.

The primary objectives were to study the impact of genetic variation in donor *CTLA-4* SNP rs4553808 on RFS (defined as survival without relapse or death), OS (defined as time to death), and nonrelapse mortality (NRM) (defined as any death without evidence of disease relapse with relapse treated as a competing risk). All data were censored at date of last follow-up. Secondary endpoints included incidence of grades 2 to 4 acute GVHD and presence of chronic GVHD. The use of ATG was considered in the analysis. An exploratory analysis of the other 9 tagSNPs (rs231775, rs231779, rs11571315, rs231777, rs3087243, rs16840252, rs231725, rs10197010, rs11571316) of *CTLA-4* was also performed to investigate potential additional associations.

Patients over the age of 18 with acute myeloid leukemia (AML) and advanced myelodysplastic syndrome (MDS) in first or second complete remission who underwent a first 8/8 or 7/8 HLA-matched unrelated donor allogeneic HSCT after any conditioning regimen between 2002 and 2007 were included in this study. HLA typing was verified using DNA-based techniques, as previously described [12]. Any GVHD prophylaxis was permitted with the exception of ex vivo T cell depletion or use of in vivo/in vitro alemtuzumab. Based on CIBMTR data, there were 1463 cases eligible for this study. A sample size calculation indicated 780 patients were required to achieve 80% power to detect a significance level (alpha) of 0.005 (increased stringency due to multiple testing) using a 2-sided log-rank test. To study the interaction with ATG, patients who received ATG were oversampled with 50% of patients studied having received ATG.

SNP analysis was performed on samples provided by the NMDP Research Repository with whole genome amplification using the REPLI-g UltraFast Mini-Kit (QIAGEN, Chatsworth, CA) and verified by RNASE-P analysis on the Taqman 7200. Genotyping of amplified DNA was performed with a custom GoldenGate (Illumina, San Diego, CA) combined with VeraCode technology on the BeadXpress according to the manufacturer's protocol (Illumina).

Each SNP was analyzed in multivariable models adjusting for clinically significant variables using the Cox's proportional hazards model. Due to multiple testing, P values of $< .005$ were considered significant. Reported P values were not adjusted for multiple comparisons. Probability of RFS and OS were calculated using the Kaplan-Meier estimator from time of transplantation. The cumulative incidence of NRM was calculated with relapse considered a competing risk. The interaction with *CTLA-4* SNPs and use of ATG was also tested with 50% of the patient population having received ATG as part of their conditioning regimen.

Table 1
Patient, Transplant, and Donor Clinical Characteristics (N = 780)

Characteristic	n (%)
Recipient age, median (range), yr	50 (18-74)
Sex	
Male	403 (52)
Female	377 (48)
Recipient race/ethnicity	
Caucasian	706 (90)
African American	22 (3)
Hispanic	29 (4)
Other/multiple/decline	23 (3)
Disease	
AML	624 (80)
MDS	156 (20)
Conditioning	
Ablative	474 (61)
Reduced intensity	222 (28)
Nonmyeloablative	84 (11)
HLA match	
8/8 matched	585 (75)
7/8 mismatch	195 (25)
GVHD prophylaxis	
Tacrolimus + MTX or MMF	480 (62)
Cyclosporine + MTX	119 (15)
Other	181 (23)
Stem cell source	
Peripheral blood	576 (74)
Bone marrow	204 (26)
HLA match	
8/8 matched	513 (66)
7/8 (1 MM at HLA-A, -B, -C, or -DRB1)	267 (34)
Donor/recipient sex match	
Male/male	276 (36)
Male/female	220 (29)
Female/male	117 (15)
Female/female	78 (21)
Donor age, median (range), yr	34 (18-61)
<20 yr	9 (1)
20-29 yr	242 (33)
30-39 yr	269 (36)
40-49 yr	174 (23)
50 and older	54 (7)
Donor race/ethnicity	
Caucasian	639 (82)
African American	25 (3)
Hispanic	23 (4)
Other/multiple/decline	93 (12)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; MTX, methotrexate; MMF, mycoohenolate mofetil; MM, mismatch; GVHD, graft-versus-host disease.

RESULTS

Table 1 lists patient-, transplantation-, and donor-related variables for patients included in the study. The final analysis included 780 patients with a median follow-up of 63 months for survivors. The median age of recipients and donors was 50 years (range, 18 to 74) and 34 (range, 18 to 61), respectively. Ninety percent (n = 706) of recipients and 82% (n = 639) of donors were of Caucasian descent. Eighty percent of patients had been diagnosed with AML (n = 624) and 20% with advanced MDS (n = 156). Conditioning was 61% myeloablative, 28% reduced-intensity, and 11% nonmyeloablative, with 74% receiving peripheral blood grafts. Seventy-five percent were HLA-matched and 25% were single-locus mismatched. Tacrolimus with methotrexate or mycophenolate mofetil GVHD prophylaxis was given to 62%, cyclosporine-based prophylaxis to 15%, and 23% received other regimens. By design, 50% of patients received ATG. Genotyping showed that 552 (71%) were AA, 208 (27%) were AG, and 19 (2%) were GG (AG, and particularly GG, were associated with worse outcomes in the prior study).

Multivariable analysis adjusting for relevant donor and recipient characteristics showed no significant association between SNP rs4553808 and OS, RFS, NRM, and the cumulative incidence of acute GVHD, chronic GVHD, and relapse (Figure 1). Multivariable analysis of the other 9 tagSNPs of the *CTLA-4* gene showed no association with primary transplantation outcomes (all *P* values > .005). No interaction was found between *CTLA-4* SNPs and use of ATG on outcomes.

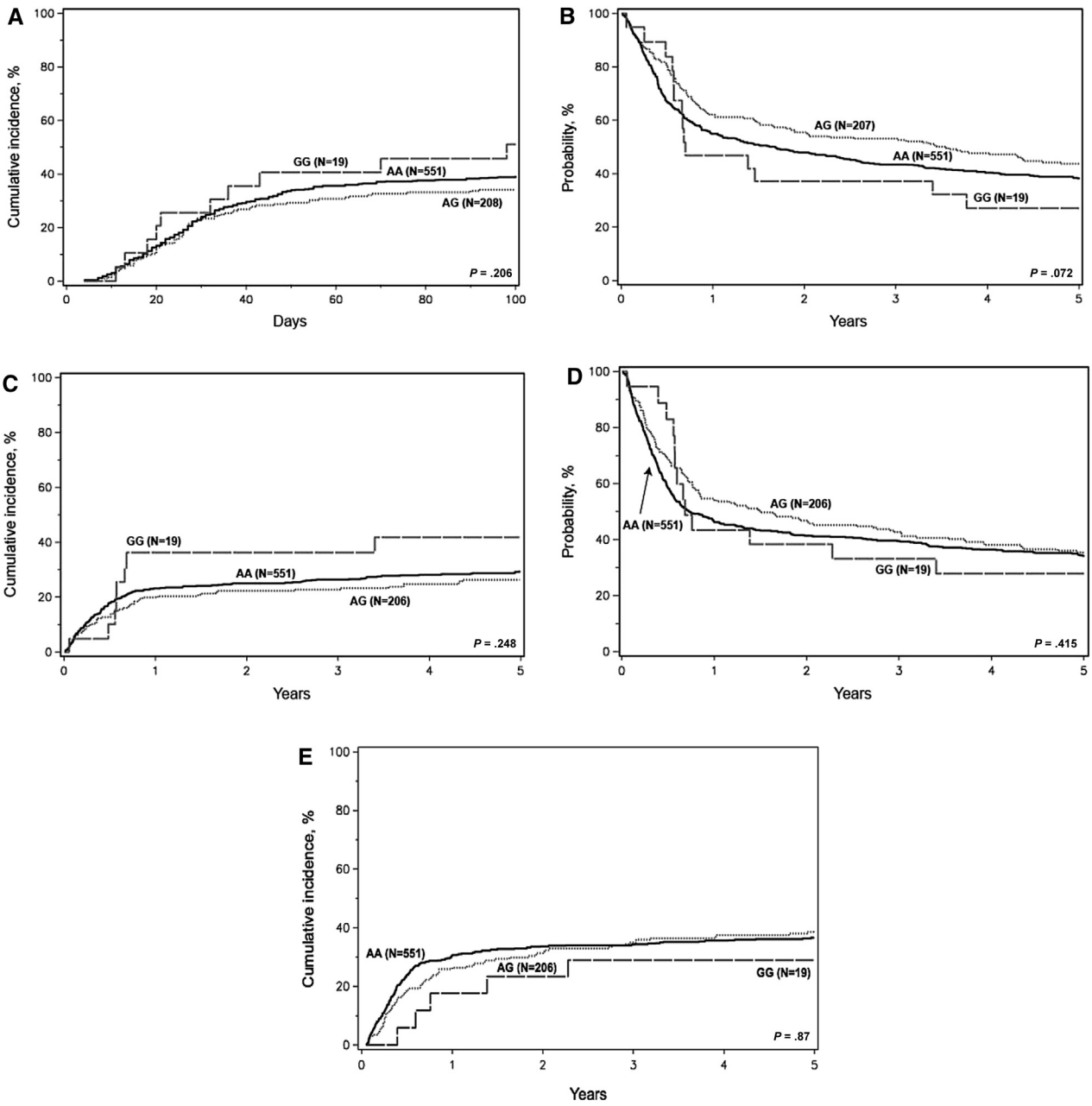

Figure 1. Clinical outcomes stratified by donor CTLA-4 SNP rs4553808 genotype. (A) shows adjusted cumulative incidence of grades 2 to 4 acute GVHD, (B) shows adjusted probability of overall survival, (C) shows adjusted cumulative incidence of nonrelapse mortality, (D) shows adjusted probability of relapse-free survival, and (E) shows adjusted cumulative incidence of relapse.

DISCUSSION

Understanding the intricate balance of T cell homeostasis in the post-transplantation period remains an important area of investigation. *CTLA-4*, an important immunologic checkpoint, and its genetic variations have been of interest in the HSCT realm because of its possible important role in immune modulation, especially as it relates to GVHD and the potency of the GVT effect. *CTLA-4* SNPs have previously been shown to be associated with RFS, OS, and incidence of GVHD [5-9], as well as a possible surrogate marker for response to donor lymphocyte infusion after HSCT [13]. This raised the possibility that a personalized approach to HSCT donor selection and conditioning regimens based on genetic variation could be realized. However, previous studies that have investigated *CTLA-4* SNPs have produced discordant results, likely because of small sample sizes and heterogeneous cohorts that, in turn, make interpretation of the true interaction of *CTLA-4* SNPS with transplantation outcomes difficult. The negative result from this present study highlights this limitation and underscores the importance of validation of preliminary genomic association studies in larger, more homogenous cohorts [14].

The strength of our study is indeed the homogeneity of our patient population with regards to the disease and transplantation type, as well as its large sample size, particularly compared with previous similar studies. To our knowledge, this study is the largest and most homogenous transplantation cohort investigating genetic variation in *CTLA-4*, although is should be noted that there were differences in other variables, such as age, degree of HLA match, conditioning regimens, and post-transplantation GVHD prophylaxis. Additionally, this large sample size allowed us to perform an exploratory analysis of other *CTLA-4* SNPs, as well as study the interaction with ATG. Our methods included increased stringency for statistical significance due to multiple testing, lending further credence to the interpretation of our results and final conclusion.

Limitations of this study include that only donor genetic variation of *CTLA-4* was studied. Although, theoretically, donor genetics likely plays a more important role in post-transplantation T cell homeostasis, there certainly could be an interaction with recipient genetic variations that were not accounted for. Secondly, because our study included only patients with AML/MDS in the unrelated donor setting, the application of our conclusion is restricted to such patients. It is possible that other disease and transplantation types may produce different results. Haplotypes of the *CTLA-4* gene were not studied as in some previous studies, and thus our conclusions are limited only to the single SNP level with regards to genetic variation. Finally, it is also possible that if an interaction is present, the impact of a single polymorphism may simply be too small to influence such expansive outcomes as NRM, RFS, and OS.

In conclusion, the observation from our previous pilot study that SNP rs3554808 was an independent predictor of transplantation outcomes was not confirmed in this larger study. Moreover, no other *CTLA-4* SNPs in our exploratory analysis were identified to be independent predictors of outcomes. Data from this study support the conclusion that donor *CTLA-4* SNPs are not associated with unrelated donor HSCT outcomes in patients with AML and MDS. Though the results of this study are negative, it should be considered definitive in such patient populations. Future studies could include investigation of genetic variation of *CTLA-4* in other disease and transplantation types, as well as other immune checkpoints, such as PD-1, especially as clinical therapeutics that target PD-1 evolve.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.03.005.

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